PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Long COVID symptoms in Israeli children with and without a history of SARS-CoV-2 infection: a cross-sectional study
AUTHORS	Adler, Limor; Israel, Moran; Yehoshua, Ilan; Azuri, Joseph; Hoffman, Robert; Shahar, Arnon; Mizrahi Reuveni, Miri; Grossman, Zachi

VERSION 1 – REVIEW

REVIEWER	Bennett, Tellen D. University of Colorado
REVIEW RETURNED	10-Jun-2022

GENERAL COMMENTS	The authors report a cross-sectional (not a cohort study, as stated in the abstract and manuscript) study of children in Israel who were part of a large HMO (2.6M citizens). The authors state that this is a nationally representative sample – a reference or evidence to support this statement would be valuable to the reader. An online questionnaire was sent by text message (Dec 2021 and January 2022) to parents of children 5-18 years old with a positive PCR for SARS-CoV-2 in the last 1-6 months (cases) and parents of children without a positive PCR (controls, 1:2 ratio). This appears to be a study of infections that occurred in the second half of 2021, so presumably the authors can only comment on prolonged symptoms related to the Delta and Omicron variants. This is a limitation that should be noted. Strengths of the study include a large sample size and a control group that in theory did not have a positive test during the same time frame. How likely is it that children in this HMO had SARS-CoV-2 tests outside this dataset? For example, were state-run testing sites available that would not be in the data (this was very common where I live)? How common was at-home testing in Israel during the second half of 2021? Weaknesses include the cross-sectional design, the potential for selection bias if parents of children with symptoms were more likely to fill out the survey, and the potential for recall bias that is differential between cases and controls. The mean 4.39 months since infection certainly raises the potential for recall bias. The range was reported as 1-12 months despite the design only going back 6 months – can the authors provide clarity on those responses? Perhaps they should be excluded. It wasn't clear to me what time frame the symptoms corresponded to – just at the time of survey completion? Page 7. Please describe the methods used, not commands in a particular statistical package. "ENTER" and "FORWARD" are not

	generalizable. Forward stepwise selection, possibly? Page 7. What statistical tests were used for comparisons of a single variable between groups?

REVIEWER	Borch, Luise Regionshospitalet Gødstrup, Child and adolescent
REVIEW RETURNED	21-Jun-2022

GENERAL COMMENTS	The manuscript is an important contribution to the knowledge on long COVID symptoms in children. As the authors state, many previous studies on long COVID in children doesn't include a control group of non-SARS-COV-2 children. This study includes a control group which is important since long covid symptoms are general symptoms that could also be prevalent in non-SARS-CoV-2 children. A study without a control group could therefore easily over-erestimate long COVID symptoms. Title: I believe the title may be a bit misleading. Children without SARS-CoV-2 infection cannot suffer from long covid symptoms. This is a concern throughout the manuscript where it's mentioned several places "long COVID symptoms in children without SARS-CoV-2 infection". This should be re-phrased. Introduction: A good introduction. The authors refer to a meta-analysis that among other thing documented that only five studies were controlled. This shows the need for more controlled studies on long covid in children. However, in line 42-50 the authors mention newer and larger studies. To my knowledge the world's largest study to date on children 0-17 years and including a control group is the study: Borch L, Holm M, Knudsen M, Ellermann-Eriksen S, Hagstroem S. Long COVID symptoms and duration in SARS-CoV-2 positive children - a nationwide cohort study. Eur J Pediatr. 2022 Apr;181(4):1597-1607. doi: 10.1007/s00431-021-04345-z. Epub 2022 Jan 9. PMID: 35000003; PMCID: PMC8742700. This study should be included in the reference list and discussed/included in the discussion as well. Methods: Well written and easy to undertand. It should be added how the authors defined long covid? Was it symptoms lasting more than 4 weeks? It is also not clear how the authors defined "long COVID" symptoms in non-SARS-CoV-2 children. Also, in the methods section there should be a referral to the complete questionnaire that was send out to the parents. The questionnaire should be included in the supplementary material. It should be mentioned if the questionnaire included questions about other infections. Results: The response rate is very low, which is a major issue regarding the validity of the results. Is 11.9% representative of a cohort of children? It is important to document the response rate separately for the two

	groups of children. If the response rate is uneven between the groups this could be an issue worth discussing. Does the 11.9% response rate cover-up for an even lower response rate in the groups separately? Was there only a 6% response rate in each group? Table 1: was there any difference between the groups (p-value)? The authors only document number and % within each group, but does not document if there was a difference in characteristics between the two groups. Discussion: Is sparse regarding limitations and how these limitations could affect the results. Limitations that is worth addressing: 1. the questionnaire is not validated. How could this affect the results? 2. How does the low response rate affect the results? 3. The fact that the symptoms reported by the parents may be symptoms from other infections than COVID. 4. Symptoms may be overestimated since it is likely that parent reported symptoms on their child who had the most symptoms In the discussion the authors compare their results to other studies. However, the present study cannot be compared 1:1 with LOngcovidkidsDK, since the Danish study only evaluated adolescents. Adolescents have been shown to have a higher prevalence of symptoms compared to younger kids. SO if the authors want to make a comparison to longcovidkidsDK study they should only compare results from adolescents. In general the authors should be aware of comparisons to other studies throughout the manuscript since many of the studies doesn't include an identical cohort when looking at age. A better reference for comparison is the study by Borch et al (mentioned above), because that study evaluates long covid symptoms in children 0-17 years. Table 3: Hearing disturbances are mentioned twice.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Tellen D. Bennett, University of Colorado Comments to the Author:
The authors report a cross-sectional (not a cohort study, as stated in the abstract and manuscript) study of children in Israel who were part of a large HMO (2.6M citizens). The authors state that this is a nationally representative sample – a reference or evidence to support this statement would be valuable to the reader.
We changed the study description throughout the manuscript.
We changed the manuscript, so it now does not say that the sample is representative. Instead, we wrote that it represents a quarter of Israel's population.

An online questionnaire was sent by text message (Dec 2021 and January 2022) to parents of children 5-18 years old with a positive PCR for SARS-CoV-2 in the last 1-6 months (cases) and

parents of children without a positive PCR (controls, 1:2 ratio). This appears to be a study of infections that occurred in the second half of 2021, so presumably the authors can only comment on prolonged symptoms related to the Delta and Omicron variants. This is a limitation that should be noted.
We added this to the limitation paragraph.

Strengths of the study include a large sample size and a control group that in theory did not have a positive test during the same time frame. How likely is it that children in this HMO had SARS-CoV-2 tests outside this dataset? For example, were state-run testing sites available that would not be in the data (this was very common where I live)? How common was at-home testing in Israel during the second half of 2021?
All formal testing (PCR / antigen) was reported and documented in the central database of each HMO. Home antigen tests were available in Israel from January 2021. However, to get a formal certificate of recovery, all people had to have a formal test. So we believe this is reliable.

Weaknesses include the cross-sectional design, the potential for selection bias if parents of children with symptoms were more likely to fill out the survey, and the potential for recall bias that is differential between cases and controls. The mean 4.39 months since infection certainly raises the potential for recall bias. The range was reported as 1-12 months despite the design only going back 6 months – can the authors provide clarity on those responses? Perhaps they should be excluded. It wasn't clear to me what time frame the symptoms corresponded to – just at the time of survey completion?
We detected children with and without a history of infection and sent their parents the questionnaire. However, we gave the parents the choice of which child to complete the questionnaire. Thus, theoretically, they can choose another child with an earlier infection. Therefore, we decided to leave these children in the study group.

Page 7. Please describe the methods used, not commands in a particular statistical package. "ENTER" and "FORWARD" are not generalizable. Forward stepwise selection, possibly?
We changed the sentence to:
"Both analyses were made using a logistic regression analysis with two blocks, the first using all variables (the ENTER approach) for baseline characteristics and forward stepwise selection for more elaborated variables."
Page 7. What statistical tests were used for comparisons of a single variable between groups?
This is mentioned in the statistical analysis:
"First, we performed univariate analysis to all long-COVID symptoms and compared children with and without a history of SARS-CoV-2 infection using the chi-square test."

Reviewer: 2
Dr. Luise Borch, Aarhus University Hospital Comments to the Author:
The manuscript is an important contribution to the knowledge on long COVID symptoms in children. As the authors state, many previous studies on long COVID in children doesn't include a control group of non-SARS-COV-2 children. This study includes a control group which is important since long covid symptoms are general symptoms that could also be prevalent in non-SARS-CoV-2 children. A study without a control group could therefore easily over-erestimate long COVID symptoms.

Title:
I believe the title may be a bit misleading. Children without SARS-CoV-2 infection cannot suffer from long covid symptoms.
This is a concern throughout the manuscript where it's mentioned several places "long COVID symptoms in children without SARS-CoV-2 infection". This should be re-phrased.
This is a good point and one that we had a long debate about. Indeed, children without SARS CoV-2 infection cannot have long-COVID. However, throughout the manuscript, we referred to long-COVID symptoms, which are very common in children and adolescents. So children without a history of infection can still experience these symptoms. In order to make it more clear to the reader, we think it should be stated as long-COVID symptoms. In the METHODS section, we added a clarification in the manuscript about this point.
"We chose to refer to these symptoms as long-COVID symptoms in both groups for clarity, although long-COVID does not exist in the control group. "

Introduction:
A good introduction. The authors refer to a meta-analysis that among other thing documented that only five studies were controlled. This shows the need for more controlled studies on long covid in children.
However, in line 42-50 the authors mention newer and larger studies. To my knowledge the world's largest study to date on children 0-17 years and including a control group is the study: Borch L, Holm M, Knudsen M, Ellermann-Eriksen S, Hagstroem S. Long COVID symptoms and duration in SARS-CoV-2 positive children - a nationwide cohort study. Eur J Pediatr. 2022 Apr;181(4):1597-1607. doi: 10.1007/s00431-021-04345-z. Epub 2022 Jan 9. PMID: 35000003; PMCID: PMC8742700.
This study should be included in the reference list and discussed/included in the discussion as well.
We added this study to our manuscript as a reference in the INTRODUCTION and the DISCUSSION.

Methods:
Well written and easy to undertand.
It should be added how the authors defined long covid? Was it symptoms lasting more than 4 weeks?
Yes. This was added to the methods section (under the subheading Variables).
It is also not clear how the authors defined "long COVID" symptoms in non-SARS-CoV-2 children.
We added clarification of this point to the methods:
" We chose to refer to these symptoms as long-COVID symptoms in both groups for clarity, although long-COVID does not exist in the control group."

Also, in the methods section there should be a referral to the complete questionnaire that was send out to the parents. The questionnaire should be included in the supplementary material.
We added the questionnaire to the supplementary material (in supplementary material – part B).

It should be mentioned if the questionnaire included questions about other infections.
The questionnaire did not include questions about other infections. Therefore, we added this to the METHODS.

Results:
The response rate is very low, which is a major issue regarding the validity of the results. Is 11.9% representative of a cohort of children?
It is important to document the response rate separately for the two groups of children. If the response rate is uneven between the groups this could be an issue worth discussing.
Does the 11.9% response rate cover-up for an even lower response rate in the groups separately? Was there only a 6% response rate in each group?

The response rate in the entire study population is 3,240/27,240 (11.9%).
Of which 1,148/8,550 (response rate of 13.4%) in children with and 2,092/18,690 (response rate of 11.2%) in children without a history of infection. Therefore, we added this to the RESULTS.

Table 1: was there any difference between the groups (p-value)? The authors only document number and % within each group, but does not document if there was a difference in characteristics between the two groups.
We added the p-value.

Discussion:
Is sparse regarding limitations and how these limitations could affect the results.
We elaborated extensively on the limitation section and addressed all points raised.

Limitations that is worth addressing:
1. the questionnaire is not validated. How could this affect the results?
2. How does the low response rate affect the results?
3. The fact that the symptoms reported by the parents may be symptoms from other infections than COVID.
4. Symptoms may be overestimated since it is likely that parent reported symptoms on their child who had the most symptoms

In the discussion the authors compare their results to other studies. However, the present study

cannot be compared 1:1 with LOngcovidkidsDK, since the Danish study only evaluated adolescents. Adolescents have been shown to have a higher prevalence of symptoms compared to younger kids. SO if the authors want to make a comparison to longcovidkidsDK study they should only compare results from adolescents. In general, the authors should be aware of comparisons to other studies throughout the manuscript since many of the studies doesn't include an identical cohort when looking at age.
A better reference for comparison is the study by Borch et al (mentioned above), because that study evaluates long covid symptoms in children 0-17 years.
We added the comparison to Borch et al. We think other comparisons are still essential and add to the discussion.

Table 3:
Hearing disturbances are mentioned twice.
We deleted the duplication.

VERSION 2 – REVIEW

REVIEWER	Borch, Luise Regionshospitalet Gødstrup, Child and adolescent
REVIEW RETURNED	26-Sep-2022

GENERAL COMMENTS	This new version of the manuscript is much better that the forst version. Especially the discussion is stronger.